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Chlorinated Insecticides in the Body Fat of People in India *

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The storage of insecticides in the body-fat of persons has previously been studied in several countries (e.g., England, France, USA) and food has been shown to be the main source from which DDT and perhaps other insecticides are absorbed. In view of the differences in dietary habits in India, a study was made to determine whether there are differences in the storage of chlorinated hydrocarbon insecticides in persons in India from that in persons in other countries.

It was found that civilians in the Delhi area with no occupational exposure to insecticides had a significantly greater deposit in their fat of DDT-related compounds and HCH than do comparable persons in the USA, but less dieldrin and heptachlor epoxide. Storage in military personnel in other Indian cities from which samples were obtained was similar to that in the USA for most DDT-related compounds and HCH but, as in Delhi, lower for dieldrin and heptachlor epoxide.

It is well known that both DDT (1,1,1-trichloro-2,2-*bis*-(*p*-chlorophenyl) ethane) and its metabolite DDE (1,1-dichloro-2,2-*bis*-(*p*-chlorophenyl) ethylene) are present in the general population of several countries (Mattson et al., 1953; Hayes et al., 1958, 1963 Hunter et al., 1963; Dale & Quinby, 1963; Hoffman et al., 1964a, 1964b). It has also been shown that dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphthalene) is present in the fat of people in the USA (Dale & Quinby, 1963; Hoffman et al., 1964a, 1964b) and southern England (Hunter et al., 1963) and that HCH (1,2,3,4,5,6-hexachlorocyclohexane) is present in the body fat of people in the USA (Dale & Quinby, 1963; Hoffman et al., 1964a, 1964b) and France (Hayes et al., 1963). It has recently been shown by the use of gas chromatography that DDD (1,1-dichloro-2,2-*bis*(*p*-chlorophenyl) ethane) and the β-isomer of HCH are stored in the body fat of the general population of New Orleans (Hayes et al., 1965). Heptachlor epoxide (1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan) has also been found in the body fat of some people in the USA (Hayes et al., 1965; Zavon et al., 1965) who had no known occupational exposure.

In India, in contrast to countries where the storage of insecticides has been studied previously, there has been nation-wide use of DDT for the control or eradication of malaria and some use in other fields of public health. DDT has not been used for preservation of grains under the control of the Government, but such preservation may be practised privately; market samples have shown residues of DDT as high as 16.3 ppm in wheat and 169 ppm in potatoes (Sharangapani & Pingale, 1954; S.K. Majumder—personal communication, 1964). HCH is widely used in plant protection and animal husbandry in India. Other chlorinated insecticides, such as dieldrin and heptachlor epoxide, find use mainly in plant protection. The dietary habits also differ in India, there being a large per capita consumption of cereals and comparatively less consumption of food derived from animal sources than in the USA and Europe. The cereals also are used in the form of whole-meal flours from which bran is generally not removed.

In countries in which the storage of insecticides has been studied previously, the diet has been shown to be the main source for the absorption of DDT (and probably other insecticides also) in persons without occupational exposure. Because of the

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dietary difference and other possible differences, it seemed desirable to learn whether there are any quantitative or qualitative differences in the storage of chlorinated hydrocarbon insecticides in people in India as compared with people in other countries.

METHODS

In order that the results of this survey might be compared with earlier surveys conducted in other countries, all the samples were analysed by the Schechter-Haller colorimetric method (Schechter et al., 1945) as modified by Mattson et al. (1953). The fat samples that were large enough were also analysed by microcoulometric gas chromatography (Coulson et al., 1960) to detect other chlorinated hydrocarbon pesticides.

The 104 samples of human fat reported on here were collected in the Dehli-New-Dehli, Amla, Jabalpur, Kanpur, Poona, and Bombay areas of India. The samples were taken from people of widely different ages (3-75 years) and occupations, all without any known occupational exposure to pesticides. In connexion with the 48 autopsy and 56 surgical samples collected, in no instance was death or illness related to pesticides. The samples were preserved in 4% formaldehyde (10% formol solution) during shipment. It has been shown (Hayes et al., 1958) that this method of preservation is satisfactory for such samples.

The samples were grouped according to place of origin and method of analysis. Group I included 69 samples from the vicinity of Delhi, all of which were analysed by the colorimetric method (Mattson et al., 1953). Twenty-four of the same samples were also analysed by microcoulometric gas chromatography (Coulson et al., 1960) and were designated as Group Ia. Group II included 19 fat samples collected in the Delhi area but from people whose addresses were not known. A total of 16 fat samples from military personnel from Amla, Jabalpur, Kanpur, Poona, and Bombay were designated as Group III and analysed by the colorimetric method; 11 of these were also analysed by microcoulometric gas chromatography and were designated as Group IIIa.

All the samples were ground and extracted with *n*-hexane in the presence of sodium sulfate. The extracts were then filtered, and those samples that were to be analysed by both the colorimetric method and microcoulometric gas chromatography were made to volume and aliquots taken for the colorimetric analysis. The remainder of the extract was cleaned up and prepared for gas chromatography (Dale & Quinby, 1963). In addition to the cleaning, this procedure gives a separation of DDE and dieldrin which could not be efficiently separated by the gas chromatographic column owing to the high concentration of DDE in the fat as compared with that of dieldrin.

The gas chromatographic analyses were carried out with a MicroTek 2500-R instrument equipped with a Dohrmann Microcoulometric Detector. The column consisted of an aluminium tube 4 feet by 1/8 inch external diameter (122 cm by 3.2 mm) packed with 1% Apiezion L and 0.2% Epikote 1001 on 80-100 mesh celite (Robinson, 1963). The temperature of the column was maintained at 130°C for the first 21 minutes after each sample was injected in order to separate the isomers of HCH. The oven temperature was then increased at a constant rate of 8.75°C per minute for four minutes. The column temperature was maintained at the attained temperature of 165°C for an additional 23 minutes for the elution of other pesticides. The carrier gas, nitrogen, was maintained at a flow rate of 26 ml/min, and the effluent gases were oxidatively decomposed at 900°C and titrated coulometrically.

RESULTS

Colorimetric analysis

Quantitative results obtained by the colorimetric method of analysis for DDT-derived materials in the fat of the general population of India are summarized in Table 1.

The mean for the total DDT-derived material in the 69 samples from Delhi was 32.1 ppm when the values for two children (a boy and a girl aged 3 and 7 years), who had DDT-derived material in their fat at concentrations of 291 ppm and 180 ppm, respectively, are considered with the values for the other 67 samples in Group I. The next highest concentration in the samples was 82 ppm. Since there was no way to exclude the possibility that exposure of the children was due to sources other than those associated with the other samples, they were not considered representative of the group and therefore were not included in the values reported in Table 1. There was no significant difference in DDE ($P > 0.70$) and DDT ($P > 0.20$) storage between Groups I and II. Although the addresses of individuals in Group II were not known, they were most likely from the Delhi area since the samples were picked up there

TABLE 1

DDT-DERIVED MATERIAL IN THE BODY FAT OF PEOPLE IN INDIA ESTIMATED BY SCHECHTER-HALLER COLORIMETRIC ANALYSIS

Group [a]	Statistical value	DDT (ppm)	DDE (ppm)	Total as DDT	DDE as DDT (%)
I	Range	2–57	<1–40	2–92	
	Mean±SE	16±1	9±1	38±2	39
	N	67	67	67	
	Group I *versus* Group III	P<0.01	P<0.05	P<0.01	
	Group I *versus* Group II	P>0.20	P>0.70	P>0.20	
Ia	Range	5–57	2–23	13–89	
	Mean±SE	18±2	11±1	30±3	41
	N	24	24	24	
II	Range	6–91	<2–26	8–118	
	Mean±SE	20±4	10±2	31±6	34
	N	19	19	19	
III	Range	1–24	<1–18	1–44	
	Mean±SE	8±2	4±1	13±3	37
	N	16	16	16	
	Group III *versus* Group II	P<0.025	P<0.025	P<0.01	
IIIa	Range	1–24	<1–16	1–44	
	Mean±SE	7±2	4±2	12±4	37
	N	11	11	11	

[a] For explanation of the groups, see the third paragraph under "Methods".

and the values are statistically similar. Samples of fat in Groups I and II contained considerably more DDT-derived material than has been reported for the general population of other countries (Table 2). The results in Table 1 show that samples from Group I contained significantly more DDE ($P<0.05$) and DDT ($P<0.01$) than those from Group III. It was also shown that there was significantly more DDE ($P<0.025$) and DDT ($P<0.025$) in Group II than in Group III.

DDT was present in greater amounts than DDE as an average for each group studied and in 60 of 67 individual fat samples of Group I, in 14 of 19 samples in Group II, and in 10 of 16 samples from Group III. DDE calculated as DDT averaged 39%, 34%, and 37% of the total DDT-derived materials in Groups I, II, and III, respectively. As shown in Table 2, these percentages are considerably lower than those reported for other countries, and the difference appears to be significant.

Since there was no significant difference in the storage of DDE and DDT in the fat of individuals in Groups I and II by the colorimetric method, these 86 individuals were considered as one group for the purpose of comparison of sex, age, autopsy *versus* biopsy, and urban *versus* rural. An analysis of variance for all ages showed that there was no significant difference ($P>0.25$) in the storage of DDT-derived materials based on age. A comparison of 41 males and 46 females showed that there was no significant difference based on sex in the storage of DDE ($P>0.05$) or DDT ($P>0.10$) in the fat. A comparison was made between 47 autopsy and 39 biopsy samples and there was no significant difference between the two groups of samples in the storage of DDE ($P>0.10$) or DDT ($P>0.50$) in the fat. There was also no significant difference in the storage of DDE ($P>0.40$) or DDT ($P>0.50$) based on a comparison of 25 urban with 16 rural fat samples from the Delhi area.

TABLE 2

CONCENTRATION OF DDT AND ITS METABOLITE DDE IN THE FAT OF THE GENERAL POPULATION OR OF CERTAIN DIETARY GROUPS OF VARIOUS COUNTRIES

Country	Year	No. of samples	DDT (ppm)	DDE as DDT (ppm)	Total as DDT (ppm)	DDE as DDT (%)	Reference
USA	1958	49	7.4	12.5	19.9	62.8	Hayes et al. (1958)
USA	1954-56	61	4.9	6.8	11.7	58.1	Hayes et al. (1958)
USA	1961-62	130	4.0	8.6	12.6	68.3	Quinby et al. (1965)
USA	1963	28	2.4	4.2	6.7	62.0	Dale & Quinby (1963)
USA	1963	382	2.9	8.2	11.1	73.9	Hoffman et al. (1964a, 1964b)
USA [a]	1955-56	16	2.3	3.6	5.9	61.0	Hayes et al. (1958)
USA [b]	1960	20	0.8	2.2	3.0	73.3	Durham et al. (1961)
Canada	1958-60	62	1.6	3.3	4.9	67.0	Read & McKinley (1961)
Germany	1958-59	60	1.0	1.3	2.3	56.5	Maier-Bode (1960)
France	1961	10	1.7	3.5	5.2	67.3	Hayes et al. (1963)
India, Group I	1964	67	16.9	10.1	26.0	39.0	This article
India, Group II	1964	19	20.3	10.7	31.0	34.0	This article
ndia, Group III	1964	16	8.1	4.7	12.8	37.0	This article

[a] Persons eating no meat.
[b] Eskimos (Alaska).

Microcoulometric gas chromatography

The system described above readily determined quantitatively 0.1 μg of a number of compounds or isomers with characteristic retention times as follows: α-HCH, 7.5; γ-HCH, 10.7; β-HCH, 16.6; δ-HCH, 19.0; heptachlor epoxide, 26.5; *o,p'*-DDE, 28.0; dieldrin, 29.4; *p,p'*-DDE, 31.6; *o,p'*-DDT, 35.5; *p,p'*-DDD, 38.6; *p,p'*-DDT, 43.4; and endrin with two peaks, 62.4 and 76.4 minutes. The α and β isomers of HCH, dieldrin, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDT were found in some or all of the 35 fat samples subjected to gas chromatography. The α-isomer of HCH was present in 30 of the 35 samples, and the β-isomer in all of them. Dieldrin was found in 24 of the 35. The *p,p'*-isomers of DDT, DDD, and DDE appeared in all samples analysed; the *o,p'*-DDT isomer was present in 21 of the 35 samples. Quantitative results obtained by gas chromatography for DDT-derived materials and other chlorinated hydrocarbons are summarized in Table 3.

Statistical evaluation of results reported in Tables 1 and 3 showed that there was no significant difference ($P > 0.80$) in the storage of total DDT-derived material in the fat based on the two methods of analysis.

Group IIIa contained significantly less *p,p'*-DDE ($P < 0.05$); *p,p'*-DDT ($P < 0.025$); and total DDT ($P < 0.025$) than Group Ia. Individuals in Group IIIa also contained significantly less α-HCH ($P < 0.001$) and β-HCH ($P < 0.001$) in their fat than those in Group Ia. There was, however, no significant difference in the storage of *p-p'*-DDD ($P > 0.02$), *o,p'*-DDT ($P > 0.10$) and dieldrin ($P > 0.20$) between the two groups.

By means of gas chromatography DDE calculated as DDT averaged 42% and 52% of the total DDT-related materials in Groups Ia and IIIa, respectively. DDD responds to the colorimetric method to give the same blue colour as that of DDT. Therefore, DDD was undoubtedly a factor contributing to the low average percentage of DDE of the total DDT-related materials found in the fat by colorimetric analysis. Furthermore, DDD is manufactured as an insecticide in its own right and, therefore, is not necessarily derived from DDT through metabolism.

TABLE 2. CONCENTRATION OF CHLORINATED HYDROCARBON PESTICIDES IN THE BODY FAT OF PEOPLE IN INDIA COMPARED WITH THAT OF PEOPLE IN NEW ORLEANS, LOUISIANA, AS MEASURED BY MICROCOULOMETRIC GAS CHROMATOGRAPHY

Location and group [a]	Statistical value	*p,p'*-DDE (ppm)	*o,p'*-DDT (ppm)	*p,p'*-DDD (ppm)	*p,p'*-DDT (ppm)	Total as DDT (ppm)	DDE as DDT (%)	α-HCH (ppm)	β-HCH (ppm)	Total as HCH (ppm)	Dieldrin (ppm)
India, Group Ia	Range	2–23	<0.08–4.1	0.5–6.5	1.3–51.3	0.90–71.5	43	<0.03–0.77	0.17–3.10	0.30–4.9	<0.02–0.26
	Mean±SE	11.6±1.2	0.70±0.26	2.7±0.4	13.9±2.4	30.2±3.8		0.22±0.07	1.30±0.17	1.7±0.28	0.09±0.01
	N	24	24	24	24	24		24	24	24	23
India, Group IIIa	Range	0.41–23.0	0.8–2.33	0.8–5.00	0.8–11.3	0.9–47.90	82	0.04–0.14	0.13–3.08	0.13–3.18	0.01–0.19
	Mean±SE	6.4±2.1	0.9±0.2	1.7±0.6	3.8±1.2	13.7±4.2		0.06±0.01	0.80±0.29	0.86±0.30	0.06±0.01
	N	11	11	11	11	11		11	11	11	11
	Group IIIa *versus* Group Ia	P<0.05	P>0.10	P>0.20	P<0.025	P<0.025		P<0.001	P<0.001	P<0.01	P>0.20
New Orleans [b]	Range	0.56–21.17	<0.01–0.67	0.09–0.99	0.15–5.99	1.1–27.09	77	<0.03	0.09–2.43	0.03–2.43	0.03–1.15
	Mean±SE	6.65±0.8	0.06±0.03	0.45±0.05	1.73±0.36	10.3 ±1.1			0.80±0.10	0.80±0.10	0.39±0.05
	N	25	25	25	25	25			25	25	25
	Group IIIa *versus* New Orleans	P>0.80	P<0.001	P<0.005	P>0.10	P>0.05			P>0.40	P>0.80	P<0.01

[a] For explanation of the groups, see the third paragraph under "Methods".

[b] The fat of the people of New Orleans also contained heptachlor epoxide (0.84±0.05 ppm) and *o,p'*-DDE (0.22±0.03 ppm). The *o,p'*-DDE is included in the total as DDT in the above tabulation.

On this basis, DDE calculated as DDT averaged 45% and 60% of the total DDT-derived materials in the fat of Groups Ia and IIIa, respectively. When calculated in a similar manner, the DDE concentration with respect to the total DDT-derived materials in the samples from the New Orleans survey mentioned below was 80%.

Regardless of any consideration of DDD, there was more DDT than *p,p'*-DDE in 13 of 24 of the samples in Group Ia. However, the absolute amount of *p,p'*-DDE stored by this group is greater than that in New Orleans (see Table 3) and, in fact, in the USA as a whole (Dale & Quinby, 1963). Since the total amount of DDT-derived material present in the fat of these individuals was greater than that of the general population of other countries (Table 2), more extensive exposure is indicated. Really high storage of DDT has been associated with low percentages of DDE. For example, formulators of insecticide preparations whose storage of DDT-related material varied from 264 ppm to 1131 ppm had only 54%-40% of it in the form of DDE (Mattson et al., 1953; Hayes et al., 1956). Furthermore, since most Indians eat little meat, they most likely would be getting a relatively low proportion of DDE in their diet, because the metabolism of DDT by animals tends to increase the proportion of DDE above that originally absorbed. Thus, both a high absolute intake of DDT and a low proportion of DDE in the diet may contribute to the fact that in India the average percentage of DDE among the total DDT-derived materials is low compared with the corresponding percentages in other countries. The high concentration of DDD in Indian samples is responsible for the fact that the proportion of DDE is lower when calculated on the basis of results obtained by the Schechter-Haller method, because by that method DDD is not measured separately but recorded as DDT.

A similar procedure of microcoulometric gas chromatography was used in a recent survey of fat from individuals of New Orleans, Louisiana. The results of that survey are included in Table 3 for comparison. Since the concentration of pesticides in the fat of the Indians in Group IIIa was equal to or lower than that of Group Ia, the statistical comparison was made between Group IIIa and the New Orleans group. Of the materials found to be present in the fat of the Indians, those in Group IIIa stored significantly less dieldrin ($P<0.01$) and significantly more *p,p'*-DDD ($P<0.005$) and *o,p'*-DDT ($P<0.001$) than people from New Orleans. There was no

significant difference between the storage of the β-isomer of HCH in the fat of Indians in Group IIIa and that in the fat of people in New Orleans. There was, however, a difference in the storage of heptachlor epoxide, which was not found in any Indian sample, and the α-isomer of HCH, which was not found in any sample from New Orleans. Since only trace amounts of the α-isomer were found in Indian fat of Group IIIa, there was no significant difference ($P<0.20$) in the storage of total HCH between this group and that from New Orleans. The total HCH stored in the fat of Indians in Group Ia (Delhi) is not significantly different ($P>0.20$) from that reported for France (Hayes et al., 1963), but is significantly greater ($P<0.001$) than that reported for the USA (Dale & Quinby, 1963). These data show that the general population of Delhi has significantly greater amounts of DDD, DDT-derived materials, and HCH but less dieldrin and less heptachlor epoxide in their fat than the general population of New Orleans. A comparison between 17 males and seven females was made for the storage of dieldrin and total HCH in the fat of Indians. There was no significant difference by sex in the storage of dieldrin ($P>0.30$) or HCH ($P>0.50$).

DISCUSSION

The frequency of high residues of DDT in samples of food taken on the open market in India is not yet known. However, some high residues have been encountered and this finding is entirely consistent with the finding that people of the Delhi area store more HCH and more DDT-related material than do people in the USA. The major remaining problem is to identify the difference in practice which accounts for the observed difference in storage in the Delhi area as compared with other cities of India from which samples were obtained. It seems possible that the difference concerns the extent of use of insecticides for the preservation of stored food in the Delhi area as compared with other cities that have been studied, or the difference may involve civilian as opposed to military practices since the samples from other cities came from soldiers or members of their families.

The finer points of difference in storage, including the presence of α-HCH and the greater concentration of *o,p'*-DDT and *p,p'*-DDD in all samples from India as compared with samples from New Orleans cannot be explained at this time.

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RÉSUMÉ

Dans différents pays, des études ont déjà été consacrées au problème de l'accumulation des insecticides dans le tissu adipeux de l'homme. Elles montrent que ces dépôts, ceux de DDT notamment, ont une origine principalement alimentaire. Les auteurs ont effectué une enquête similaire en Inde afin de rechercher une influence éventuelle des habitudes alimentaires des populations de ce pays sur l'accumulation dans l'organisme des hydrocarbures chlorés.

Au cours de nécropsies ou d'interventions chirurgicales, 104 échantillons de tissu adipeux humain ont été prélevés chez des personnes de 3 à 75 ans, non exposées professionnellement à un risque inhabituel d'absorption d'insecticides. L'examen de ces préparations a été effectué par la méthode colorimétrique ou par chromatographie gazeuse. Il a permis de déceler la présence des α- et β-HCH, de la dieldrine, de l'isomère *p,p'* du DDD, des isomères *o,p'* et *p,p'* du DDT, de même que celle de l'isomère *p,p'* du DDE. L'analyse d'échantillons prélevés parmi la population civile de Delhi montre une teneur significativement plus élevée en α- et β-HCH et en composés du groupe DDT que dans les fragments de tissu adipeux provenant de personnes appartenant au personnel militaire vivant dans d'autres régions de l'Inde. On ne relève aucune différence importante entre les taux de l'isomère *p,p'* du DDD et de la dieldrine dans la popu-

lation de Delhi et celle d'autres régions du pays. En revanche, le tissu adipeux des habitants de la région de Delhi contient moins de dieldrine que celui de certaines populations des Etats-Unis d'Amérique, mais sa teneur en HCH et en composés du groupe DDT est plus élevée. A Delhi et dans d'autres régions de l'Inde, le DDE intervient pour 42% et 52% respectivement dans l'ensemble des substances du groupe DDT. Cette proportion est très nettement inférieure à celle que l'on observe aux Etats-Unis (62%); les Indiens, consommant moins de viande, accumulent relativement moins de DDE que les populations d'autres pays. Il est possible que les habitants de l'Inde soient exposés à de plus fortes concentrations de DDD; les Indiens de Delhi sont certainement exposés à de plus fortes concentrations de DDT et d'HCH que la population d'autres pays.

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